Transactions of the Royal Society of S. Aust. (1999), **123**(2), 69-75.

A COMPARISON OF SOME SOIL MICROINVERTEBRATE ASSEMBLAGES IN SOUTHERN AUSTRALIA

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Summary

BIRD, A.F. (1999) A comparison of some soil microinvertebrate assemblages in southern Australia. *Trans. R. Soc. S. Aust.* **123**(2), 69-75, 31 May, 1999.

Microinvertebrates from five widely diverse environments have been isolated and living specimens examined. A total of 24,237 organisms was counted. They consisted of annelids, archiannelids, crustaceans, insects, molluscs, nematodes, tardigrades and turbellarians. In all instances nematodes predominated as follows: edge of lake numbers (n) 86%, taxa (t) 79%, ocean beach n 53%, t 76%, river bank n 87%, t 71%, river estuary n 93%, t 84% and wheat field n 91%, t 87%. The mean percentage of nematodes as numbers (n) and taxa (t) in these soils was n=82 and t=79.

The numbers of nematodes per litre of soil at each site ranged from 80-17,300 and the numbers of taxa from 11-21, although some were classified only to class or phylum. These results clearly indicate the abundance, richness and dominance of nematodes compared with other soil microinvertebrates in these widely diverse habitats. Reasons for the relatively low overall counts are discussed.

KEY WORDS: Microinvertebrates, nematodes, diverse environments, abundance, biodiversity, meiofauna.

Introduction

Earlier research into the microinvertebrates of South Australian soils has indicated that nematodes predominate in all soil environments studied (Nicholas *et al.* 1992; Yeates & Bird 1994). However, no quantitative comparisons with other micrometazoans over a range of habitats have previously been made. Where quantitative comparisons between groups of animals have been made, such as on the macroinvertebrates at Goyder Lagoon (Sheldon & Puckridge 1998), it is possible to establish the degree of dominance. In this study, insects dominated making up 63% of individuals and 76% of taxa. These organisms were collected at the soil surface by sweeping with a fine mesh net. However, separation of microinvertebrates from the soil is more complex and typically involves either sieving through a range of sieves or utilizing movement in response to gravity in a misting apparatus (Yeates & Bird 1994).

Within the soil, microscopic nematodes are known to be as biodiverse as the macroinvertebrates above it (Lawton *et al.* 1998) and are considered to be the most abundant metazoans (Bernard 1992).

The principle objective of the work reported here was to quantify the abundance and diversity of the main taxonomic groups of soil-inhabiting microinvertebrates in a range of environments.

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Materials and Methods

Soil samples were collected from five different environments. All of these soils are classified under the US soil classification (Soil Survey Staff 1998) as Entisols or young sandy soils. One of these was terrestrial (a wheat field) and is subclassified as an orthent with the texture of a dry sandy loam. The remaining four were semi-aquatic from the shore of a lake, the edge of a river, the shore of an estuary and an ocean beach. All of these were wet sands and were classified as aquents.

Terrestrial environment (1)

(1) Samples were collected on 20 April 1998 from sandy loam soil at a site (34° 14' S, 138° 19' E) near Avon, SA. This site had been direct drilled and had a wheat/wheat rotation. The soil was moist after rain which had fallen the previous week and which had broken the summer drought. Soil was sampled to a depth of 11.5 cm using a 4.7 cm diameter corer thus giving a sample volume of approximately 200 ml. Ten samples were collected at regular intervals giving a final soil volume of 2 l which was mixed in a plastic bag and stored in a polystyrene box for transport back to the laboratory.

Within several hours of its collection the soil sample was sieved through a 2 mm sieve, weighed into 50 g aliquots and placed in a misting machine for four days as described previously (Yeates & Bird 1994). The misting process both aerates the soil and stimulates movement of the micrometazoa which

TABLE 1. *List of sites, their localities and environmental characteristics.*

No. (see Fig. 1)	Sites Name	GPS Reading	Soil classification (US)	% Clay	% Silt	% Sand Fine	% Sand Coarse	Texture	Salinity Total Soluble Salts mg l⁻¹
1	Wheat field (Avon)	lat 34° 14' S long 138° 19' E	Entisol - orthent	12	2	7	79	Sandy loam	*nd
2	Lake Alexandrina	lat 35° 23' S long 139° 03' E	Entisol - aquent	<1	<1	<1	99	Sandy	300
3	Glenelg River (Dartmoor)	lat 37° 55' S long 141° 17' E	Entisol - aquent	nd	nd	nd	nd	Sandy	1800
4	River Murray estuary (Goolwa)	lat 35° 32' S long 138° 50' E	Entisol - aquent	<1	<1	32	67	Sandy	23 500
5	Ocean beach (Guichen Bay)	lat 37° 10' S long 139° 45' E	Entisol - aquent	0	0	1	99	Sandy	34 200

* nd = not determined

gravitate through the soil and into the collecting tubes. At the completion of the extraction and after sedimentation and supernatant removal, the living micrometazoa were counted following the method of Bird (1996) and classified into major groups.

Aquatic environments (2-5)

The remaining four environments were considered to be aquatic since all the soils were water-logged and merging with the water's edge. They were all sandy soils and the micrometazoa were extracted by sieving. In sequence of increasing salinity the soils were:

(2) Lake Alexandrina at the mouth of the Bremer River (35° 23' S, 139° 03' E). Collected 26 August 1998. The lake was choppy and almost covered the sandy beach where the collection was made. The Bremer River had partly flooded the area of rushes and reeds adjacent to the lake.

(3) Glenelg River at Dartmoor (Vic.) near Fort O'Hare and just before the junction with the Crawford River (37° 55' S, 141° 17' E). Collected 29 July 1998 after heavy rain.

(4) River Murray estuary between the sea and the seaward side of the Goolwa barrage (35° 32' S, 138° 50' E). Collected 2 June 1998.

(5) Ocean beach at Guichen Bay at Robe (37° 10' S, 139° 45' E). Collected 16 September 1998 in the intertidal zone with the tide receding. The ocean was calm.

In each case five samples were collected using the 4.7 cm corer giving a total volume of approximately 1 l. The soil was mixed in a bucket with water from the environment being studied. The water was free of microinvertebrates as determined initially by eye and later by microscopic examination. The soil was sieved through 2 mm, 800 µm and 750 µm sieves and then material was collected on 500 µm, 300 µm, 125 µm, 75 µm and 53 µm sieves. The material was washed from these sieves into a beaker and decanted into 200 ml tissue culture flasks. The contents of the flasks were tipped into counting chambers and allowed to gravitate. The living micrometazoa were then examined and counted under a dissecting microscope and classified into major groups using bright field and differential interference contrast optics.

Soil sections

Soil samples were taken by the method described by Brewer & Sleeman (1988) and were transported to the laboratory in an ice box. They were freeze dried in the laboratory and impregnated with araldite *in vacuo* (Cent & Brewer 1971). After polymerization, thin sections, ranging in thickness from 20-40 µm, were cut using a diamond tipped saw blade and were then ground on a rotary diamond lap.

These sections were examined and photographed under polarized light with an Olympus Vanox photomicroscope using Ilford Delta 400 film.

Results

The environments

The environmental characteristics and locations for the five sites are given (Table 1, Fig. 1). The sites are widely separated, ranging from a wheat field with a sandy loam textured soil to wet sandy soils from fresh water habitats situated on the banks of Lake Alexandrina and the Glenelg River, respectively, to saline habitats at a river estuary and a sandy beach. The salinities of these environments range from 300 mg l⁻¹ for the shore of Lake Alexandrina to 34,200 mg l⁻¹ for the ocean beach at Guichen Bay.

Microinvertebrate assemblages

A total of 24,237 individuals from approximately 93 taxa was counted from the five samples. Some

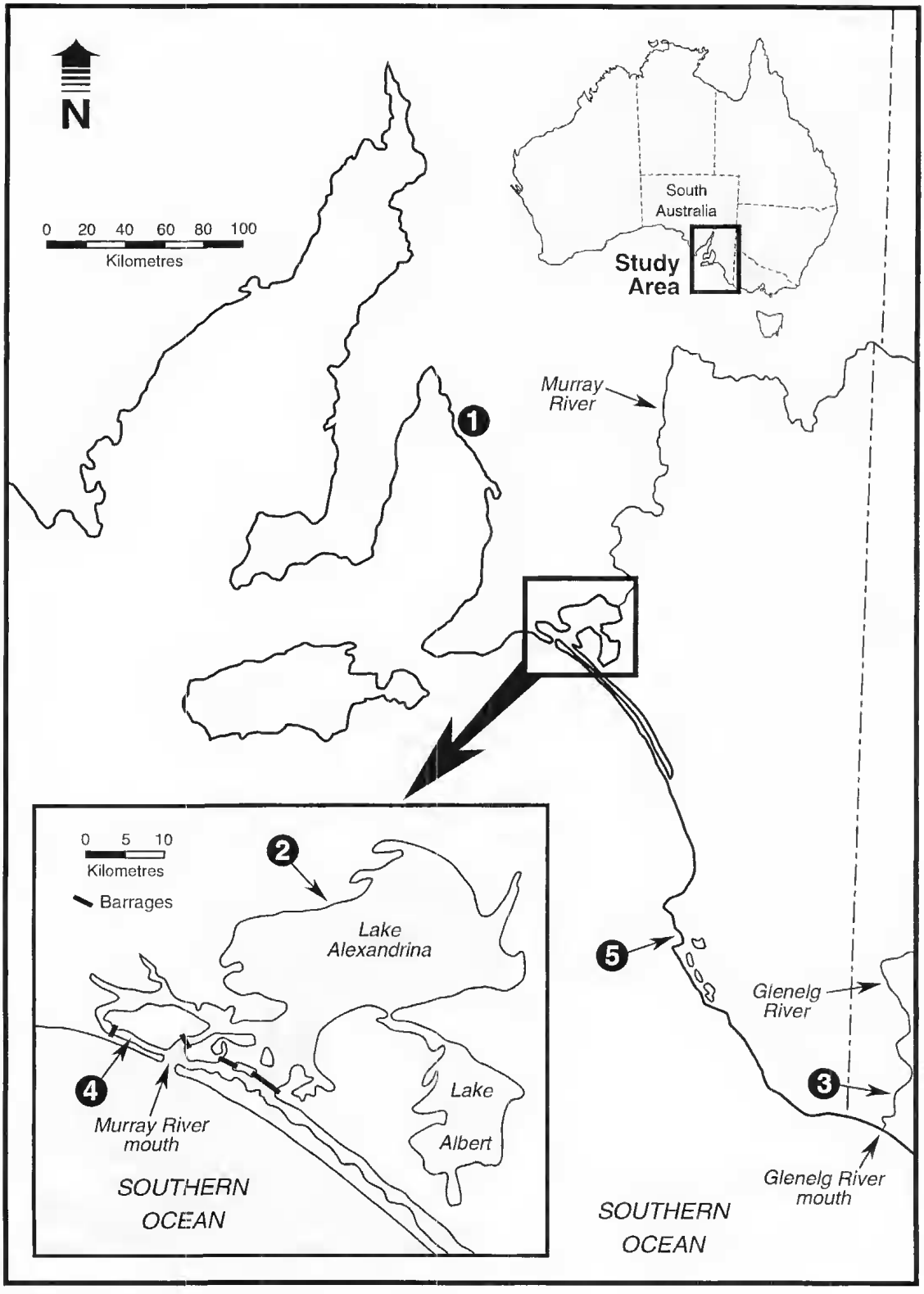

Fig. 1. Maps showing collecting sites.

TABLE 2. *Microinvertebrate numbers (n) and major taxonomic groups (t) extracted from soil sample cores taken to a depth of 11.5 cm in five widely dispersed geographic localities in southern Australia.*

Zoological groups	Localities (1) Wheat field (Avon)	(2) Lake Alexandrina	(3) Glenelg River (Dartmoor)	(4) River Murray estuary (Goolwa)	(5) Ocean beach (Guichen Bay)
Nematodes					
n (nos l⁻¹ soil)	1400	690	17300	1800	80
as % fauna	91	86	87	93	53
t (taxonomic groups)	13	11	17	21	13
as % taxa	87	79	71	84	76
Archiannelids					
n (nos l⁻¹ soil)	-	-	-	-	33
as % fauna	-	-	-	-	22
t (taxonomic groups)	-	-	-	-	1
as % taxa	-	-	-	-	6
Other Annelids					
n (nos l⁻¹ soil)	12	50	1900	48	12
as % fauna	0.6	6	10	2.5	8
t (taxonomic groups)	1	1	3	1	1
as % taxa	6.5	7	13	4	6
Turbellarians					
n (nos l⁻¹ soil)	-		170	66	
as % fauna	-	-	1	3	-
t (taxonomic groups)	-		1	1	-
as % taxa	-	-	4	4	
Tardigrades					
n (nos l⁻¹ soil)	130	30	40	-	-
as % fauna	8.3	4	0.2	-	
t (taxonomic groups)	1	1	1	-	-
as % taxa	6.5	7	4	-	
Insects					
n (nos l⁻¹ soil)	-	30	390	-	
as % fauna	-	4	2	-	-
t (taxonomic groups)	-	1	2		-
as % taxa	-	7	8	-	-
Crustaceans					
n (nos l⁻¹ soil)	-	-	-	29	25
as % fauna	-	-	-	1.5	16
t (taxonomic groups)	-	-	-	2	1
as % taxa	-	-	-	8	6
Molluscs					
n (nos l⁻¹ soil)	-		-	-	2
as % fauna	-	-	-	-	1
t (taxonomic groups)	-	-	-	-	1
as % taxa	-	-	-	-	6

specimens were identified to species and some of these occurred in more than one of the five environments. Other specimens could only be placed in families or orders. Nematodes were the dominant group comprising 82% of individuals and 79% of taxa (Table 2). The numbers of nematodes per litre of soil at each site ranged from 80 at the ocean beach site to 17,300 at the Glenelg River bank and the number of taxa from 11 on the bank of Lake Alexandrina to 21 for the River Murray estuary. It must be emphasized that figures for these taxa are only approximate due to a combination of limited taxonomic knowledge, rapidity of assessment and some replication of taxa. These limitations are discussed below.

In the wheat field 91% of the numbers of animals counted were nematodes and they comprised 87% of the taxa. Tardigrades made up most of the remainder representing just over 8% of the animals. They consisted entirely of *Macrobiotus* cf. *pseudohufelandi* Iharos 1966 (Bird 1996; Bird & McClure 1997). Tardigrades were also found to a lesser extent in the wet sandy soils of the Glenelg River and Lake Alexandrina shores and belonged to a different family. Nematodes comprised 87% and 86% of the numbers and 71% and 79% of the taxa, respectively, in these environments (Table 2). Annelids made up 10% of the numbers of the microinvertebrates of the Glenelg River bank, the remaining organisms comprising insect dipteran larvae (2%) and an unidentified species of turbellarian (1%). A thrip insect, identified as *Frankliniella schultzei* (Trybom) (A. Wells pers. comm. 1998) made up 4% of the Lake Alexandrina assemblage together with a

species of annelid (6%) and a species of tardigrade (4%). In addition, a large number of copepod and cladoceran Crustacea was found swimming in the water above the soil but these were not considered to be part of the soil environment.

In the more saline wet soils of the River Murray estuary below the Goolwa barrages and at Guichen Bay, nematodes constituted 93% of the numbers and 84% of the taxa for the former and 53% of the numbers and 76% of the taxa in the latter. Both of these environments contained small annelids and those from the river estuary were identified as genera belonging to the family Naididae (K. Lee pers. comm. 1998). These were the only environments with Crustacea in the soil samples rather than in the water. The ocean beach sample was the only one to contain molluscs (1% of the numbers and 6% of the taxa) and an archiannelid (22% of the numbers and 6% of the taxa). The archiannelids resemble the genus *Polygordius* and lack setae or parapodia. Because of their enigmatic appearance, they are listed separately here from the other annelids (Table 2).

Soil sections

It is difficult to recognize and classify organisms in soil sections although soil sections do give some idea of the environment in which these microinvertebrates have to move and feed. Thus, a 20 µm vertical section through the saline wet sandy soil (aquent) of the Murray River estuary and photographed under bright field optics (Fig. 2) shows part of a nematode that is 40 µm wide and is surrounded by sand grains ranging from about 50 µm to 300 µm in diameter and which exhibit birefringence under polarized light, interspersed with some darker coloured organic material. This soil contains about 180 nematodes 100 ml^{-1} (Table 2) so that the chance of obtaining easily identifiable microinvertebrates from tangential soil sections is remote.

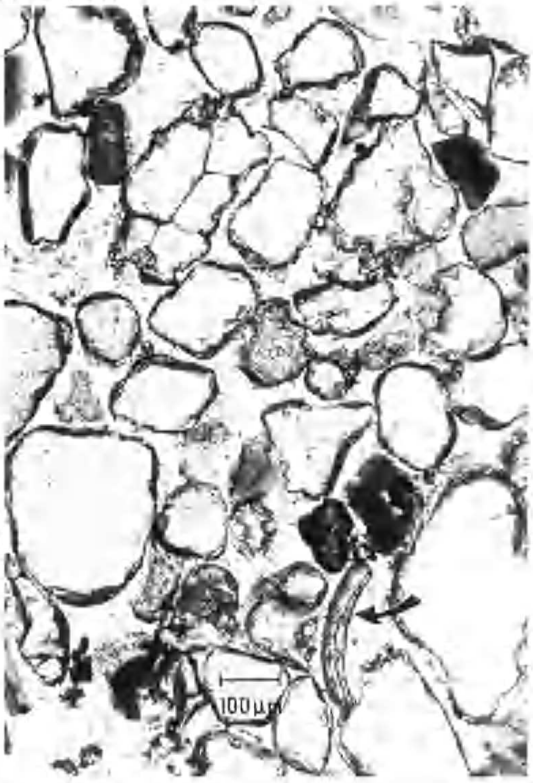

Fig. 2. A 20 µm thick vertical section cut through the top 0.5–2.5 cm of soil at the River Murray estuary, collected at site 4, see Fig. 1. Photographed under bright field optics and showing part of a nematode (arrow).

Discussion

It is clear from these results that nematodes predominate both in numbers and diversity among the micrometazoa in a wide range of soil environments. Just as insects can predominate among the macroinvertebrates at the soil surface (Sheldon & Puckridge 1998) nematodes predominate among the microinvertebrates within the soil. Their numbers vary depending on the time of the year that they are collected and the weather conditions on the day of collection. Thus, in the wheat field soil at Avon, there are many more nematodes present when the wheat and weeds are growing during winter (Yeates & Bird 1994), as indicated by the presence of plant parasitic forms, than at the end of summer when there is only dry stubble on the ground and few, if any, plant parasitic forms. Similarly, it has been shown (Nicholas *et al.* 1992) that nematode numbers on the shore of Lake Alexandrina vary markedly from month to month throughout the year. When the lake is rough or during the hottest months of January, February and March, there is considerable mortality of nematodes and other microinvertebrates as judged by the presence of dead specimens during counting (pers. obs). Also, there was an increase in nematode mortality when the salinity in the River Murray estuary dropped following the opening of the barrage gates and the discharge of River Murray water (Nicholas *et al.* 1992).

It seems that climatic and seasonal variations as well as human interference can cause measurable changes in nematode population numbers. However, these changes seem to influence all the micrometazoa since the percentage of nematodes in these populations remains constant. Thus the percentage of nematodes present in the micro-

metazoan population of a Lake Alexandrina sample collected on 3.ii.1998 was 87 compared with 86 for a sample collected six months later on 26.viii.1998, although there was a three-fold difference in nematode numbers (pers. obs.).

The proportion of nematodes to other microinvertebrates in the five different environments examined was uniformly high, ranging from 53-93% with a mean of 82%. Furthermore, nematodes were the only microinvertebrate group, apart from annelids, to occur in all the environments studied and had much greater diversity than any other group (Table 2). Because of their relatively low numbers in the environments studied, other micrometazoa may have been present but not detected. For example, tardigrades were present in one collection (26.viii.1998) from the shores of Lake Alexandrina but not in another (2.ii.1996). Similarly, some forms may occur in large numbers in the water over the soil but not in the soil as was the case with Crustacea (copepods and cladocerans) on one occasion (26.viii.1998) at the Lake Alexandrina site.

The archiannelids recovered from the ocean beach at Robe were small (0.35-1.95 mm long), were covered with cilia and had a pair of anterior lateral tentacles (or cirri). They appeared to exude a sticky mucus. Differences in size might have been due to damage caused by sieving since the anterior parts of all specimens examined seemed to have similar dimensions e.g. the tentacles in all samples measured were about 150 µm long and 10 µm wide. Thus the shorter specimens might have been broken during collection.

Nematodes have been recognized as the most abundant metazoans in the soil (Bernard 1992) but, although there is general agreement on this point, quantitative comparisons with other groups of micrometazoa in various different environments are rare. Raffaelli (1982) compared the numbers of six microinvertebrate groups, namely nematodes, copepods, turbellarians, archiannelids, enchytraeids and gastrotrichs from 17 sandy marine beaches around Great Britain. Calculations from his Table 2 show that nematodes averaged 75% of organisms in all these sites. Similarly, McLachlan's (1985) work on the fauna of sandy beaches in Western Australia showed that nematodes are the most abundant of the meiofauna. Calculation of the means from the eight sites given in his Table 4 show that nematodes made up 56% of the microinvertebrates, crustaceans 24%, annelids 10%, turbellarians 7% and other groups 3%. It is interesting to note that although nematodes and crustaceans (harpacticoids) were present in all the beach sites examined by McLachlan (1985), annelids (oligochaetes) were absent from four of the eight sites and turbellarians from two of them. However, if comparisons between different investigations are to be made, accurate and uniform sampling methods need to be adopted. As Lawton *et al.* (1998) point out, in their work on biodiversity in a tropical forest, a vast amount of effort is required in compiling an inventory of the organisms present and this applies particularly to microscopic organisms.

It is agreed by some (Ladd *et al.* 1981) that the biomass of an organism in the soil is more important than its numbers, particularly when determining the labile nitrogen and carbon content of the soil. A factor that is sometimes not taken into account, although it is particularly important, is the reproductive capability of the organism in question. Because some soil nematodes can complete their life cycles in three days and each female can lay several hundred eggs within a couple of weeks, the number can grow to millions (Bird & Bird 1991) with a greater biomass than much larger and more slowly reproducing forms. In nature these huge increases in number are kept in check by a range of factors such as competition, predation and limited food resources. Thus, huge numbers of nematodes are rarely, if ever, found in nature, with the above-mentioned factors being responsible, at least in part, for the variations in actual numbers that can occur at different times at the one site. For example, Nicholas & Hodda (in press) found that the numbers of nematodes at a given sandy beach site can vary considerably, being lowest in winter and highest during the summer. However, the proportions of nematodes to the other microinvertebrate phyla in the soil appear to remain remarkably constant.

It seems reasonable to ask ourselves what factors the Insecta and Nematoda share that give them the competitive edge in attaining dominance in their respective environments. A major factor may be their ability to moult which provides a mechanism for their transition into or out of a resistant abiotic stage in which their metabolism almost comes to a standstill.

Four of the six major groups mentioned above, namely, the Insecta, Crustacea, Tardigrada and Nematoda are thought to be phylogenetically related and, together with some less abundant groups, the onycophorans, nematomorphans, kinorhynchs and priapulids, have been grouped into a clade called Ecdyozoa which emphasizes their common ability to undergo ecdysis or moult (Aguinaldo *et al.* 1997). The concept that moulting arose only once is put forward for further testing (Aguinaldo *et al.* 1997). It remains to be seen whether or not this monophyly of moulting animals is confirmed by later investigators.

In conclusion, this paper is an attempt to draw attention to the numbers of free living nematodes in a range of soil environments. The numbers counted are lower than those that actually occur because of the limitations of the techniques employed in their

isolation and detection, particularly as only living and moving material was considered. Furthermore, the number of taxa counted was limited by the author's knowledge of nematode taxonomy. However, all material was fixed and preserved for subsequent identification.

These preliminary studies emphasize the need to examine the microinvertebrates of the soil in more detail and to understand further the ecology of the nematodes that dominate in these environments. This is a largely unexplored area of research that has been overlooked by those involved in research on soil microbiology.

Acknowledgments

I wish to thank J. Bird for constructive criticism of the manuscript and for assisting with collecting. CSIRO Land & Water provided accommodation, facilities and expertise, including that of A. Beech (water analyses), J. Coppi (collecting), R. Fitzpatrick and W. Hudnell, a visiting scientist from USA (advice on soils), K. Lee (annelid identification) and G. E. Rinder (mapping). I should also like to thank T. Cribb, University of Queensland and M. Currini-Galletti (visitor to the British Museum) (archiannelid identification) and A. Wells, ABRS Canberra (thrip identification). This research was made possible by a grant from the Australian Biological Resources Study.

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